REMARKS

I. Status of the Application

Claims 1, 3-6, 8, 10-12, and 14-21 are pending in the present application. No claims are currently being amended or cancelled. The Claim Listing is merely provided here for the Examiner's convenience. Claims 1, 4, 6, 8, 11, 14, 16, 18, and 20 stand rejected under 35 U.S.C. § 102(b) over U.S. 5,100,651 ("Boyer"). Claims 1, 3-6, 8, 10-12, and 14-21 stand rejected under 35 U.S.C. § 112, ¶1 as not being enabling. Applicant traverses each rejection in view of the following remarks.

II. Claims 1, 4, 6, 8, 11, 14, 16, 18, and 20 Are Patentable Over Boyer

Claims 1, 4, 6, 8, 11, 14, 16, 18, and 20 stand rejected under 35 U.S.C. §102(b) over Boyer for reasons set forth in the Office Action dated March 29, 2001 and in the Office Action dated February 20, 2003. Furthermore, the Examiner believes that the bromochlorophene of Boyer meets the requirement of Applicant's claimed cationic antimicrobials. The Examiner bases this conclusion on a comparison of the alexidene and the chlorhexidine salts (i.e., chlorhexidine diacetate and chlorhexidine digluconate) disclosed in the present specification with Boyer's bromochlorophene. The Examiner concludes that if the alexidene and chlorhexidine salts are cationic, then so is Boyer's bromochlorophene.

In response, Applicant is presenting herewith evidence that alexidene and chlorhexidine are considerd by one of skill in the art as being cationic antimicrobials just as Applicant represents in the specification.

Also, Applicant thoroughly explained (and demonstrated with chemical structures) in the previous Amendment and Response dated May 9, 2003 that Applicant's cationic antimicrobials

are in fact cationic and Boyer's bromochlorophene is not cationic. In fact, bromochlorophene is charge neutral. The Examiner has identified no objective evidence of record or scientific reasoning to support his belief that bromochlorophene is a cationic antimicrobial. In particular, Applicant provided the chemical structures of certain cationic antimicrobials listed in claim 4 (i.e., cetylpyridinium chloride, domiphen bromide, benzalkonium chloride, and benzethonium chloride). The Examiner does not presently appear to deny that the aforementioned antimicrobials are cationic. Rather, the Examiner's present objection appears to be limited to the remaining antimicrobials listed, e.g., in claim 4 (i.e., alexidene, chlorhexidine diacetate, and chlorhexidine digluconate).

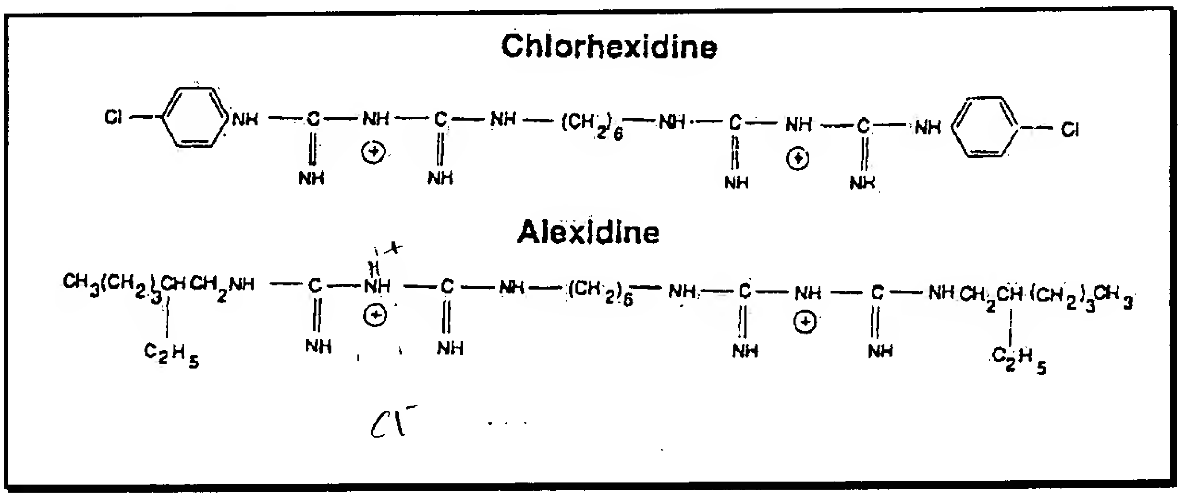
Alexidene, chlorhexidine diacetate, and chlorhexidine digluconate are indeed cationic antimicrobials, which can be bound to a negatively charged surface by virtue of their ability to carry a positive charge. Applicant explains the significance of cationic antimicrobials to the mechanism of action of the presently disclosed inventions at page 4, lines 15-24:

> An embodiment of the invention describes a formulation that utilizes a proteinaceous animal chew such as rawhide, and a dentally therapeutic **cation**, (in this example, chlorhexidine) that is **maintained on the surface of the chew on the basis of charge attraction.** The cationic antimicrobials become strongly bound to negatively charged surfaces containing negatively charged moieties such as carboxylic, phosphate and sulfate moieties by forming salt bridges. Cationic antimicrobials that are released from the carrier in the presence of saliva are observed to have a long duration of action, due to **their retention and adherence to the negatively-charged surface** in the oral cavity, e.g., enamel hydroxyapatite, acquired pellicle protein, and the oral mucosa. (Emphasis added).

It is readily apparent from Applicant's disclosure that Applicant's cationic antimicrobials, including alexidene, chlorhexidine diacetate, and chlorhexidine digluconate, coordinate with or bind to a negatively charged surface on the basis of opposite charge attraction.

Further, it is apparent from their structures that alexidene, chlorhexidine diacetate, and chlorhexidine diacetate are cationic. Alexidene and chlorhexidine are both bisguanidines having structures shown at Tab A (Merck Index 11th ed., No. 222 and Merck Index 11th ed., No. 2090, respectively). Those of skill in the art appreciate that the guanidino nitrogens of alexidene and chlorhexidine carry a positive (cationic) charge. For example, Mitchell *et al.* at Tab B show alexidene and chlorhexidine having positive charges on their respective guanidino nitrogens, the structures of which are reproduced in Figure 1 for the Examiner's convenience.

Figure 1: Chlorhexidine and Alexidene



Apparently, by virtue of their bisguanidine structures, alexidene and chlorhexidine carry a positive (cationic) charge that allows them to coordinate to anions or negatively charged species.

Further, those of skill in the art refer to alexidene and chlorhexidine as cationic antimicrobials despite the presence of a counterion (such as diacetate or digluconate) to help solubilize the free base. For instance, Gjermo states, "Chlorhexidine is a bis-biguanide formula with cationic properties." See Tab C. Moreover, Wagle acknowledges in U.S. 6,660,716 at

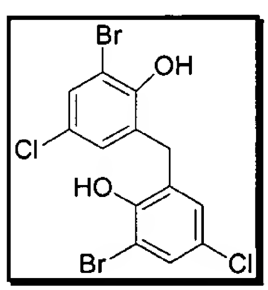
column 24, lines 7-12 (reproduced below) that alexidine, cetyl pyridinium chloride, chlorhexidine gluconate, hexetidine, and benzalkonium chloride are cationic. See Tab D.

> Recently, a class of cationic anti-microbial agents with remarkable anti-plaque properties have been formulated in oral rinses for regular use to kill bacteria in the mouth. These agents, the **cationic antiseptics, include** such agents as **alexidine,** cetyl pyridinium chloride, **chlorhexidine gluconate,** hexetidine, and benzalkonium chloride. (Emphasis added).

Still, others in the art know alexidene and chlorhexidine as cationic antimicrobials. For example, in an authoritative textbook, chlorhexidine is referred to as being cationic. See Graham W. Denton, *Chlorhexidine* in *Disinfection, Sterilization, and Preservation*, 274 (Seymour S. Block ed., 1991) at Tab E. Thus, Applicant's antimicrobials, alexidene, chlorhexidine diacetate, and chlorhexidine digluconate, are indeed cationic.

In stark contrast, Boyer's bromochlorophene is not a cationic compound, like alexidene, chlorhexidine digluconate, and chlorhexidine diacetate. Indeed, bromochlorophene is a charge neutral compound that is not capable of carrying a positive charge because bromochlorophene does not have any nitrogen atoms that can be protonated to provide a positive charge. As discussed in the previous Amendment and Response dated May 9, 2003, bromochlorophene is known by those of skill in the art as being a charge neutral compound, as evidenced by its chemical structure shown in Figure 2.

<u>Figure 2: Bromochlorophene</u>



At best, bromochlorophene, being a bisphenol, may be an *anionic* compound that can have a negative charge on a carbon atom (to form a carbanion) or on an oxygen atom (to form a phenoxide). Most importantly, Boyer does not disclose or otherwise teach or suggest (and the Examiner has not shown with evidence) that bromochlorophene is cationic, which it is not. Thus, bromochlorophene is not a cationic antimicrobial.

Thus, Applicant respectfully requests removal of the present rejection and allowance of all claims at this time.

III. Claims 1, 3-6, 8, 10-12, and 14-21 Satisfy 35 U.S.C. § 112, ¶1

Claims 1, 3-6, 8, 10-12, and 14-21 stand rejected under 35 U.S.C. § 112, ¶1 as not being enabling. Applicant respectfully traverses this rejection.

The Examiner asserts at page 2 of the Office Action that "Applicant's assertions and claims are inconsistent with Applicant's arguments [presented in the Amendment and Response dated May 9, 2003]." The Examiner further asserts "the claimed compounds are not cationic compounds. . . so it is not now evident how one maintains and obtains the compounds and the carrier in cationic and negatively charged form respectively." Applicant respectfully disagrees with this assertion.

At the outset, contrary to the Examiner's assertion, Applicant's assertions and claims are indeed consistent with Applicant's earlier presented arguments. Applicant has thoroughly explained that the claimed antimicrobials are cationic. Clearly, cetylpyridinium chloride, domiphen bromide, benzalkonium chloride, and benzethonium chloride are cationic, as indicated in the previous Amendment and Response dated May 9, 2003. The Examiner does not appear to deny this. Likewise, alexidene, chlorhexidine diacetate, and chlorhexidine digluconate are

cationic as evidenced by their structures and the discussion immediately above. Thus, all of the presently claimed antimicrobials are cationic.

The above-referenced claims are enabled by the specification insofar as Applicant discloses cationic antimicrobial compounds throughout the specification. For example, Applicant discloses at page 5, lines 9-11:

> Cationic antimicrobials contemplated to have utility in the invention include chlorhexidine diacetate, chlorhexidine digluconate, cetylpyridinium chloride, domiphen bromide, benzalkonium chloride, benzethonium chloride, and alexidene.

Applicant has established above and in the previous Amendment and Response dated May 9, 2003 that all of the above-referenced compounds are cationic. That is, all of the above-referenced compounds are capable of carrying a positive charge. Accordingly, the cationic antimicrobials have the ability to coordinate with or bind to a negatively charged surface, as explained above. See page 4, lines 15-24 (quoted above). Applicant also provides examples of how to make and use the presently claimed devices and methods at pages 5-6. Thus, those of skill in the art given the benefit of this disclosure would readily understand how to make and use the claimed devices and methods. Regarding the carrier, Applicant discloses throughout the specification, for example at page 4, lines 2-5, that the carrier is negatively charged:

> According to the invention, a carrier is utilized that has a negative surface charge. The carrier may be formed from natural or synthetic substances, and further may be inherently negatively charged, or may be coated by a reagent that imparts the negative charge to the surface of the carrier.

Apparently, Applicant teaches those of skill in the art how the carrier is negatively charged, how the cationic antimicrobials are positively charged, and how the cationic antimicrobials are positioned close to or at the surface of the carrier. Thus, all pending claims are enabled by the specification and removal of the present rejection is respectfully requested at this time.

IV. Conclusion

Having addressed all outstanding issues, Applicant respectfully requests reconsideration and allowance of all pending claims at this time.

Respectfully submitted,

Dated: October 7, 2003

John P. Iwanicki, Reg. No. 34,628
BANNER & WITCOFF, LTD.
28 State Street, 28th Floor
Boston, MA 02109
617-720-9600

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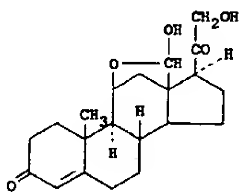
18,21-diol; Aldocorten; Aldocortin; Electrocortin. $C_{21}H_{28}O_5$; mol wt 360.44. C 69.97%, H 7.83%, O 22.20%. Adrenocortical steroid which exerts regulatory influence on metabolism of electrolytes and water. Isoln: Simpson *et al.*, *Experientia* **9**, 333 (1953); *Helv. Chim. Acta* **37**, 1163 (1954); Mattox *et al.*, *J. Am. Chem. Soc.* **75**, 4869 (1953); Harman *et al.*, *ibid.* **76**, 5035 (1954). Solutions contain an equilibrium mixture of the aldehyde and the hemiacetal, the equilibrium favoring the latter. Structure: Tait *et al.*, *Experientia* **10**, 132 (1954); *Helv. Chim. Acta* **37**, 1200 (1954). Crystal structure and molecular conformation: Duax, Hauptmann, *J. Am. Chem. Soc.* **94**, 5467 (1972). ^{13}C-NMR spectrum: P. Gerard, *Org. Magn. Resonance* **11**, 478 (1978). Total synthesis: Schmidlin *et al.*, *Helv. Chim. Acta* **40**, 1438 (1957); Johnson *et al.*, *J. Am. Chem. Soc.* **80**, 2585 (1958); **85**, 1409 (1963). Three-step synthesis from corticosterone: Barton, Beaton, *ibid.* **82**, 2640 (1960); **83**, 4083 (1961). Alternate synthesis: D. H. R. Barton *et al.*, *J. Chem. Soc., Perkin Trans. I* **1975**, 2243; M. Miyano, *J. Org. Chem.* **46**, 1846 (1981). Biosynthesized in the zona glomerulosa and transported chiefly by albumin. In man, 400 μg secreted normally in one day. Secretion influenced by ACTH, growth hormone, plasma sodium and potassium, and the renin-angiotensin system. Causes reabsorption of Na^+, Cl^-, and HCO_3^- and diuresis of K^+. *Review:* L. F. Fieser, M. Fieser, *Steroids* (Reinhold, New York, 1959) pp 701-720.



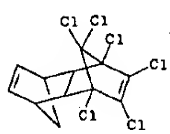
Hydrated crystals from dilute acetone, mp 108-112° (when anhydr mp 164°). $[\alpha]_D^{23}$ +152.2° (anhydr; c = 2 in acetone). $[\alpha]_D^{25}$ +161° (c = 0.1 in chloroform). uv max: 240 nm (log ε 4.20 for the monohydrate; ϵ_{mol} 15,000 for the anhydr).

21-Acetate, $C_{23}H_{30}O_6$, flat needles from acetone + ether, mp 198-199°, $[\alpha]_D^{24}$ +121.7° (c = 0.71 in chloroform). Synthesis: Wettstein *et al.*; Jeger, U.S. pats. **3,002,972** and **3,014,029** (both 1958 to Ciba).

Minimum observable activity of the free alcohol in the urinary sodium retention assay occurs at between 0.05 and 0.01 γ per rat, while at least 16 γ of desoxycorticosterone acetate is required for the same level of activity.

THERAP CAT: Mineralcorticoid.
THERAP CAT (VET): Mineralcorticoid.

219. Aldrin. *1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4:5,8-dimethanonaphthalene;* HHDN; compd 118; Octalene. $C_{12}H_8Cl_6$; mol wt 364.93. C 39.50%, H 2.21%, Cl 58.30%. Activity: C. W. Kearns *et al.*, *J. Econ. Entomol.* **42**, 127 (1949). Prepn of aldrin and *endo,endo*-isomer: Lidov, U.S. pat. **2,635,977** (1953 to Shell). Alternate syntheses: Schmerling, U.S. pat. **2,911,447** (1959 to Universal Oil Prod.); Korte, Rechmeier, *Ann.* **656**, 131 (1962).



Crystals, mp 104°. Vapor press at 20°: 7.5 × 10^{-5} mm Hg. Very sol in most organic solvents and insol in water. Stable in presence of organic and inorganic alkalies; stable to the action of hydrated metal chlorides. LD_{50} orally in male, female rats: 39, 60 mg/kg, T. B. Gaines, *Toxicol. Appl. Pharmacol.* **14**, 515 (1969).

endo,endo-Isomer, ***isodrin, compd 711.*** Crystals, mp 240-242°. LD_{50} orally in male, female rats: 15, 7.0 mg/kg, T. B. Gaines, *loc. cit.*

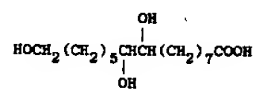
Caution: Poisoning may occur by ingestion, inhalation, skin absorption. Severe symptoms may result from ingestion or percutaneous absorption of 1 to 3 g, especially in presence of liver disease. *Acute toxicity:* Renal damage, tremors, ataxia, convulsions followed by CNS depression, respiratory failure, death. *Chronic toxicity:* Prolonged exposure may cause hepatic damage, *cf.* Patty's *Industrial Hygiene and Toxicology* vol. **2B**, G. D. Clayton, F. E. Clayton, Eds. (Wiley-Interscience, New York, 3rd ed., 1981) pp 3702-3707.

USE: Formerly as insecticide; manuf and use has been discontinued in the U.S.

220. Aletris. Star grass; starwort; true unicorn root; blazing star; colic root. Rhizome of *Aletris farinosa* L., *Liliaceae. Habit.* Eastern U.S., Ontario. *Constit.* Starch, diosgenin. Isoln of sapogenin: Marker *et al.*, *J. Am. Chem. Soc.* **62**, 2620 (1940). Pharmacological studies: Butler, Costello, *J. Am. Pharm. Assoc., Sci. Ed.* **33**, 177 (1944).

THERAP CAT: Antiflatulent.

221. Aleuritic Acid. DL-*erythro-9,10,16-Trihydroxyhexadecanoic acid;* 9,10,16-trihydroxypalmitic acid; 8,9,15-trihydroxypentadecane-1-carboxylic acid. $C_{16}H_{32}O_5$; mol wt 304.42. C 63.12%, H 10.60%, O 26.28%. One of the constituent acids of shellac. Obtained in 43% yield from dewaxed shellac: Schaeffer, Gardner, *Ind. Eng. Chem.* **30**, 333 (1938); Gidvani, *J. Chem. Soc.* **1944**, 306; Sengupta, Bose, *J. Sci. Ind. Res. (India)* **11B**, 458 (1952). The acid obtained from shellac is optically inactive, although it contains two asymmetric carbon atoms. It has been shown to be the DL-*erythro* or *dl-cis* form, and is the only form described here. Synthesis of diastereoisomers: Mitter *et al.*, *Sci. Cult. (Calcutta)* **8**, 273 (1942); Hunsdiecker, *Ber.* **76**, 142 (1943); **77**, 185 (1944); Baudart, *Compt. Rend.* **221**, 205 (1945).



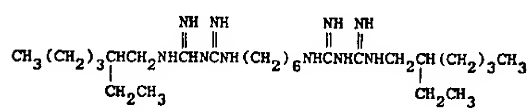
Crystals from dilute ethanol, mp 100-101°. Sol in methanol. Forms a crystalline sodium salt.

Methyl ester, $C_{17}H_{34}O_5$, fine feathery needles, mp 72-73°; $bp_{0.1}$ 235°. Sol in methanol, ethanol, chloroform, acetone. Less sol in benzene. Insol in petr ether.

Ethyl ester, $C_{18}H_{36}O_5$, needles from dil ethanol, mp 59°.

Hydrazide, $C_{16}H_{34}N_2O_4$, crystals from abs ethanol, mp 139-140°.

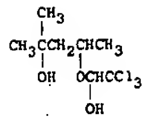
222. Alexidine. *N,N''-Bis(2-ethylhexyl)-3,12-diimino-2,4,11,13-tetraazatetradecanediimidamide; 1,1'-hexamethylenebis[5-(2-ethylhexyl)biguanide];* Win 21904; Sterwin 904; Bisguadine. $C_{26}H_{56}N_{10}$; mol wt 508.81. C 61.37%, H 11.09%, N 27.53%. Prepn from 1,1'-hexamethylenebis(3-cyanoguanide) and 2-ethylhexylamine hydrochloride: Fr. pat. **1,463,818** (1965 to Sterling Drug). Evaluation as antimicrobial agent: McNamara *et al.*, *J. Soc. Cosmet. Chem.* **16**, 499 (1965).



Dihydrochloride, crystals from methanol + ether, mp 220.6-223.4°.

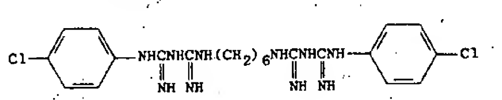
THERAP CAT: Antibacterial.

223. Alexitol Sodium. Sodium polyhydroxyaluminum monocarbonate hexitol complex; aluminum sodium carbonate hexitol complex; Actal. Probable structure and properties: Gwilt *et al.*, *J. Pharm. Pharmacol.* **10**, 770 (1958). Stabilization of an antacid prepn contg gelatinous aluminum hydroxide with a hexitol (sorbitol or mannitol): Alford, U.S. pat. **2,999,790** (1962 to Sterling Drug).



Crystals, slightly bitter taste, mp 102-104°. Readily sol in alcohol, chloroform; moderately sol in ether; slightly sol in CCl_4. Hydrolyzes in aq soln.
Caution: May be habit forming. This is a controlled substance (depressant) listed in the U.S. Code of Federal Regulations, Title 21 Part 1308.13 (1985).
THERAP CAT: Hypnotic.

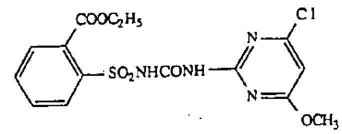
2090. Chlorhexidine. *N,N''-Bis(4-chlorophenyl)-3,12-diimino-2,4,11,13-tetraazatetradecanediimidamide; 1,1'-hexamethylenebis[5-(p-chlorophenyl)biguanide];* 1,6-bis[N'-(*p*-chlorophenyl)-N^5-biguanido]hexane; 1,6-bis(N^5-*p*-chlorophenyl-N'-diguanido)hexane; 1,6-di(4'-chlorophenyldiguanido)hexane; 10040; Nolvasan; Sterilon. $C_{22}H_{30}Cl_2N_{10}$; mol wt 505.48. C 52.28%, H 5.98%, Cl 14.03%, N 27.71%. Bisbiguanide with bacteriostatic activity. Prepn: Rose, Swain, *J. Chem. Soc.* **1956**, 4422; *eidem,* U.S. pat. **2,684,924** (1954 to I.C.I.). Antibacterial activity and acute toxicity: G. E. Davies *et al., Brit. J. Pharmacol.* **9**, 192 (1954). Review of toxicology and clinical uses: D. M. Foulkes, *J. Periodont. Res.* **8**, Suppl. 12, 55-60 (1973). Series of articles on clinical efficacy in gingivitis and plaque control: *ibid.* **21**, Suppl. 16, 1-89 (1986).



Crystals from methanol, mp 134°. Strong alkaline reaction. Soly in water at 20°: 0.08% (w/v).
Dihydrochloride, $C_{22}H_{32}Cl_4N_{10}$, *Lisium.* Crystals, dec 260-262°. Soly in water at 20°: 0.06 g/100 ml.
Diacetate, $C_{26}H_{38}Cl_2N_{10}O_4$, *Chlorasept 2000.* Crystals, mp 154-155°. Neutral reaction. Soly in water at 20°: 1.9 g/100 ml. Aq solns dec when heated above 70°. Soluble in alcohol, glycerol, propylene glycol, polyethylene glycols. LD_{50} orally in mice: 2 g/kg (Davies).
Digluconate, $C_{34}H_{54}Cl_2N_{10}O_{14}$, *Bacticlens, Corsodyl, Hibiclens, Hibidil, Hibiscrub, Hibitane, Orahexal, Peridex, pHiso-Med, Plac Out, Plurexid, Rotersept, Unisept.* Soly in water at 20°: > 50% (w/v). LD_{50} in mice (mg/kg): 22 i.v.; 1800 orally (Foulkes).
THERAP CAT: Topical antibacterial; disinfectant.
THERAP CAT (VET): Topical and uterine antiseptic; disinfectant.

2091. Chloric Acid. $ClHO_3$; mol wt 84.46. Cl 41.98%, H 1.19%, O 56.83%. $HClO_3$. Prepd from barium chlorate and sulfuric acid: Lamb *et al., J. Am. Chem. Soc.* **42**, 1643 (1920); from sodium chlorate using ion-exchange resins: Klement, *Z. Anorg. Allgem. Chem.* **260**, 271 (1949).
Known in aq soln only. Aq solns are stable if pure and protected from light. 1% aq soln d_4^{18} 1.0044; 6% soln d_4^{18} 1.0344; 10% soln d_4^{18} 1.0594; 16% soln d_4^{18} 1.0991; 20% soln d_4^{18} 1.1273; 24% soln d_4^{18} 1.1568. A 40% soln corresponds to $HClO_3.7H_2O$, d_4^{20} 1.282. If higher concns are attempted by evaporation the soln begins to dec with evolution of chlorine and oxygen and formation of perchloric acid. The salts of chloric acid are known as chlorates.
USE: Oxidizing agent; with H_2SO_3 as catalyst in acrylonitrile polymerization. *Caution:* Strongly irritating to skin, mucous membranes.

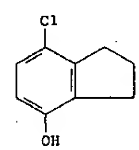
2092. Chlorimuron Ethyl. *2-[[[[(4-Chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid ethyl ester;* ethyl 2-[[[[(4-chloro-6-methoxypyrimidin-2-yl)-amino]carbonyl]amino]sulfonyl]benzoate; DPX-F6025; Classic. $C_{15}H_{15}ClN_4O_6S$; mol wt 414.82. C 43.43%, H 3.64%, Cl 8.55%, N 13.51%, O 23.14%, S 7.73%. Selective sulfonylurea herbicide. Prepn: A. D. Wolf, Austrian pat. **8,316,181**; *idem,* U.S. pat. **4,547,215** (1984, 1985 both to Du Pont). Effect on plant growth and pigment synthesis: R. M. Devlin, Z. K. Koszanski, *Proc. Ann. Meet. Northeast. Weed Sci. Soc.* **40**, 115 (1986). Metabolism by plants: H. M. Brown, S. M. Neighbors, *Pestic. Biochem. Physiol.* **29**, 112 (1987). Field trial in soybeans: G. N. Rhodes *et al., Tenn. Farm Home Sci.* **142**, 21 (1987). HPLC determn in crops: J. L. Prince, R. A. Guinivan, *J. Agr. Food Chem.* **36**, 63 (1988). Brief review: J. S. Claus, *Weed Technol.* **1**, 114-115 (1987).



Crystals from butyl chloride, mp 198-201°. Soly (ppm): acetone 71000, acetonitrile 31000, benzene 8000, methylene chloride 153000, water (pH 7) 1200, (pH 6.5) 450, (pH 5) 11. LD_{50} in male, female rats (mg/kg): 4102, 4236 orally (Claus).
USE: Herbicide.

2093. Chlorinated Lime. Bleaching powder. Improperly called "*chloride of lime*" or "*calcium oxychloride*". A relatively unstable chlorine carrier in solid form; a complex chemical compd of indefinite composition, presumably consisting of varying proportions of $Ca(OCl)_2$, $CaCl_2$, $Ca(OH)_2$ and H_2O in its molecular structure. Maximum available chlorine content approaches 39%. Commercial products usually range between 24% and 37% of available chlorine.
White or grayish-white powder; strong odor of chlorine. On exposure to air it becomes moist and rapidly decomposes. Most of it dissolves in water or alcohol. *Keep dry and tightly closed.*
Human Toxicity: Strong solns irritate skin. Inhalation of fumes may cause laryngeal and pulmonary irritation, pulmonary edema, death. Ingestion may produce severe oral, esophageal, gastric irritation.
USE: Bleaching of wood pulp, linen, cotton, straw, oils, soaps, and in laundering; oxidizer in calico printing to obtain white designs on a colored ground; destroying caterpillars; disinfecting drinking water, sewage, etc.; as a decontaminant for mustard gas and similar substances.
THERAP CAT: Disinfectant.
THERAP CAT (VET): Disinfectant for premises. Has been used as a topical antiseptic for superficial wounds.

2094. Chlorindanol. *7-Chloro-2,3-dihydro-1H-inden-4-ol; 7-chloro-4-indanol.* C_9H_9ClO; mol wt 168.63. C 64.10%, H 5.38%, Cl 21.03%, O 9.49%. Prepn: Buck *et al., J. Am. Chem. Soc.* **79**, 3559 (1957); Buck, U.S. pat. **2,990,-324** (1961 to Esta Med. Labs.).



Needles from petr ether, mp 91-93°. Absorption spectra: Buck *et al., loc. cit.* Ingredient of *Lanesta.*
USE: Spermaticide.

2095. Chlorine. Cl; at. wt 35.453; at. no. 17; valences 1 to 7; elemental state: Cl_2. A halogen. Abundance in igneous rock (95% of earth's crust): 0.031% by wt; in seawater: 1.9% by wt (primarily as NaCl). Natural isotopes: 35 (75.53%); 37 (24.47%); seven radioactive isotopes and two isomers are known; radioactive tracer elements: ^{36}Cl ($T_{1/2}$ 3.08 × 10^5 yrs; β^-, EC); ^{38}Cl ($T_{1/2}$ 37.29 min; β^-); formed in atm by bombardment with cosmic rays. Discovered in 1774 by Scheele; recognized as an element in 1810 by Davy. Produced on a large scale by electrolysis from fused chlorides. The industrial product is about 99.3% pure. Contaminants are traces of bromide, hexachloroethane, hexachlorobenzene, and water. Purification: Fye, Beaver, *J. Am. Chem. Soc.* **63**, 1268 (1941); A. Klemenc, *Die Behandlung und Reindarstellung von Gasen* (Vienna, 2nd ed., 1948) p

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QacA Multidrug Efflux Pump from *Staphylococcus aureus*: Comparative Analysis of Resistance to Diamidines, Biguanidines, and Guanylhydrazones

BERNADETTE A. MITCHELL, MELISSA H. BROWN, AND RONALD A. SKURRAY*

School of Biological Sciences, University of Sydney, New South Wales 2006, Australia

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The staphylococcal multidrug efflux pump QacA mediates resistance to a broad spectrum of monovalent and divalent antimicrobial cations. Resistance toward various classes of these compounds identified features of the substrate that may be important for interaction with QacA. Analysis of combinations of two substrates suggested that the same mechanism is used for the extrusion of different classes of compounds.

The plasmid-encoded multidrug resistance gene *qacA* from *Staphylococcus aureus* mediates resistance to a number of classes of antimicrobial organic cations, including intercalating dyes, quaternary ammonium compounds, diamidines, and biguanidines (3, 10). *qacA* has been shown to encode a protein, QacA, with 14 transmembrane segments (6, 9) that confers resistance via export of the compound energized by the proton motive force (3). QacA is a member of the major facilitator superfamily of transport proteins, which are involved in the uniport, symport, and antiport of a wide range of substances across the cell membrane (4, 7). A closely related protein, QacB, also from clinical isolates of *S. aureus*, characteristically differs from QacA in that it mediates significantly reduced levels of resistance to divalent cationic drugs, such as diamidines and biguanidines (3, 6). Random and site-directed mutagenesis showed that the difference in substrate specificity between QacA and QacB is due to a single amino acid substitution at position 323, where the presence of an acidic residue in QacA is essential for high levels of resistance to diamidines and biguanidines (6).

A series of diamidine variants was used to establish if the difference in substrate specificity between QacA and QacB exists for a wide range of structures. The various diamidine structures enabled the effects of altered interamidine linkage (x) and the addition of side chains (y) to be examined (Fig. 1A). The diamidine amicarbalide differs from those shown in Fig. 1A by the position of the amidine groups attached to the aromatic rings. MIC analysis demonstrated that QacA conferred significantly higher levels of resistance than did QacB for all of the diamidines tested, irrespective of the interamidine linkage, the addition of side chains, or the position of the amidine group on the aromatic ring (Table 1). These results are consistent with the hypothesis (6) that the negative charge of the acidic amino acid at position 323 in transmembrane segment 10 of QacA interacts directly with one of the positively charged moieties of the divalent cation and, as such, may be involved in substrate binding or recognition, resulting in increased QacA-mediated export of these compounds.

The biguanidines and guanylhydrazones are structurally related to the diamidines (Fig. 1). These compounds represent different chemical classes that all have the common feature of amidine groups attached to aliphatic or aromatic structures. The guanylhydrazones contain the amidine (guanyl) group attached to the hydrazone group, and the biguanidines possess the guanido in place of the amidine group. QacA and QacB have previously been shown to differ in their substrate specificity for the biguanidine chlorhexidine (3). However, phenotypic analysis showed that neither QacA nor QacB conferred resistance to alexidine, which represents an aliphatic derivative of chlorhexidine, or to chlorguanide, a monovalent derivative of chlorhexidine (Table 1). MIC analysis revealed that both QacA and QacB conferred resistance to divalent aromatic guanylhydrazones but not to the divalent aliphatic guanylhydrazone methylglyoxal-bisguanylhydrazone (Table 1) or to trivalent guanylhydrazones (data not shown). Notably, the characteristic difference in substrate specificity of QacA and QacB for the diamidines was absent for the divalent aromatic guanylhydrazones, indicating that at least one amino acid common to both proteins is involved in conferring resistance to these compounds. Phenotypic analysis of different salts of the guanylhydrazones and chlorhexidine confirmed that the nature of the anionic component of the salt is relatively unimportant (Table 1), reiterating the view that the Qac proteins interact with a solubilized cationic substrate. In addition, the cationic amidine moiety may not in itself be sufficient for interaction with the Qac proteins but may need to be attached to or contained within an aromatic ring to facilitate recognition and transport of these compounds. Unlike some other multidrug efflux proteins in the major facilitator superfamily which can recognize both anionic and cationic substrates (7), neither QacA nor QacB conferred resistance to anionic substances such as hydrophilic (enoxacin and norfloxacin) or hydrophobic (nalidixic acid) quinolones (data not shown).

To further examine the hypothesis that QacA-mediated resistance to structurally dissimilar compounds is conferred via a common mechanism, fractional inhibitory concentration (FIC) analysis of various combinations of two QacA substrates was performed. Monovalent-divalent combinations were represented by ethidium-propamidine and benzalkonium-propamidine. Divalent-divalent combinations were represented by propamidine-pentamidine and propamidine-chlorhexidine. Increasing increments of each compound were combined by the checkerboard procedure (8) as follows: 200 to 800 μg/ml for ethidium, 20 to 80 μg/ml for benzalkonium, 50 to 300 μg/ml for pentamidine and propamidine, and 1 to 12 μg/ml for chlorhexidine. Microtiter plates and 20-ml agar plates were incubated for 48 h at 37°C. FIC index = $x/\mathrm{MIC}(x) + y/\mathrm{MIC}(y)$, where x and y

* Corresponding author. Mailing address: School of Biological Sciences A12, University of Sydney, Sydney, New South Wales 2006, Australia. Phone: (61)(2)-9351-2376. Fax: (61)(2)-9351-4771. E-mail: skurray@bio.usyd.edu.au.

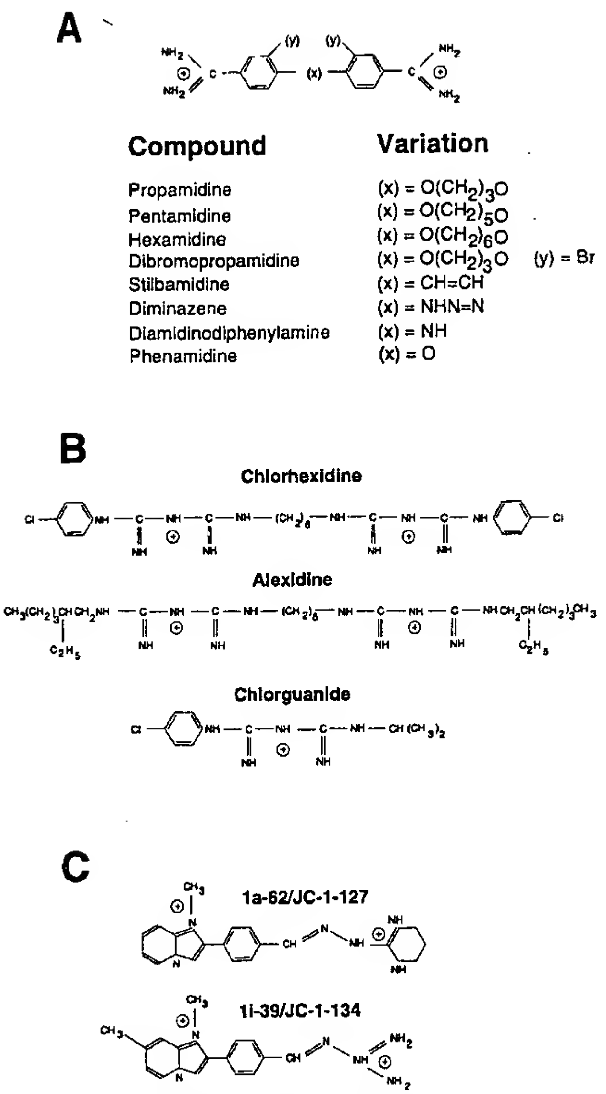

FIG. 1. Chemical structures of diamidines, biguanidines, and guanylhydrazones used in this study. (A) Diamidines: structural variations at x (interamidine linkage) and y (side chains). (B) Biguanidines: chlorhexidine (digluconate and dihydrochloride) and alexidine represent aromatic and aliphatic biguanidines, respectively. Chlorguanide is a monovalent derivative of chlorhexidine. (C) Guanylhydrazones: 1a-62, JC-1-127, 1i-39, and JC-1-134 are divalent aromatic guanylhydrazones. 1a-62 and JC-1-127 have the same structure but represent dibromide and dichloride salts, respectively (11). 1i-39 and JC-1-134 vary in the position of a methyl side chain in that 1i-39 is 1,7-dimethyl (shown) and JC-1-134 is 1,6-dimethyl; they also represent dibromide and dichloride salts, respectively (11). Structures were taken from information provided by suppliers.

represent the lowest concentration of each compound (in combination) at which there is no growth and MIC(x) and MIC(y) represent the individual MIC of each compound. FIC indices have been defined as follows: ≤0.50, synergy; 0.51 to 2.00, additivity; 2.01 to 4.00, indifference; and >4, antagonism (1, 8). In this study, we have adapted the FIC index based on the assumption that if QacA extrudes two different compounds via the same mechanism, then an additive result would be expected, whereas if distinct mechanisms for different chemicals operate independently, then the result would be one of indifference. The results for all combinations tested fell within a

TABLE 1. Resistance to diamidines, biguanidines, and guanylhydrazones

Compound[a]	MIC (μg/ml)[b]		
	QacA	QacB	Control
Biguanidines			
Alexidine	6	4	4
Chlorguanide	250	250	250
Chlorhexidine[c]	12	6	1
Diamidines			
Amicarbalide	1,200	400	200
Diamidinodiphenylamine	250	50	50
Dibromopropamidine	10	1	1
Diminazene	400	200	200
Hexamidine	300	200	100
Pentamidine	350	200	100
Phenamidine	1,800	200	200
Propamidine	300	100	100
Stilbamidine	400	200	100
Guanylhydrazones			
1i-39/JC-1-134[d]	>2,000	1,600	100
1a-62/JC-1-127[d]	1,600	1,600	500
Methylglyoxal-bisguanylhydrazone	1,200	1,200	1,200

[a] Amicarbalide, chlorguanide, diamidinodiphenylamine, dibromopropamidine, phenamidine, propamidine, and stilbamidine were obtained from Rhône Poulenc Rorer (Dagenham, United Kingdom); hexamidine was obtained from Chauvin Laboratoire (Montpellier, France); and the guanylhydrazones, JC-1-127, JC-1-134, 1a-62, and 1i-39 (11), were obtained from Richard Sundberg (University of Virginia, Charlottesville).

[b] MICs were determined in triplicate with *Escherichia coli* K-12 strain BHB2600 (2) carrying plasmids pSK4219 (*qacA*) and pSK4270 (*qacB*) and the vector pBluescript (control) (6).

[c] Chlorhexidine dihydrochloride and digluconate gave equivalent results.

[d] Similar results were obtained for the different salts (see Fig. 1 legend) of these compounds.

range of FIC indices from 0.67 to 1.33, consistent with an additive effect.

The data presented in this report, together with those from previous studies (3, 5, 10), demonstrate that the multidrug efflux protein QacA is able to confer resistance to more than 30 cationic lipophilic antimicrobial compounds that belong to 11 distinct chemical classes. No resistance was observed for trivalent cationic substances or anionic substances, indicating that the resistance specificity of QacA is restricted to monovalent and divalent cationic substrates. Furthermore, FIC analysis of combinations of two substrates from various chemical classes showed an additive result, suggesting that QacA operates via a single antiport mechanism for the export of structurally dissimilar lipophilic cationic substrates.

We thank R. Sundberg, Rhône-Poulenc Rorer and Chauvin Laboratoire, for the generous supply of chemicals used in this study.

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CHLORHEXIDINE

Gjermo, P. Chlorhexidine in dental practice. J. Clin. Periodontol. 1:143, 1974.

Purpose:

This is a review article describing chlorhexidine and its practical uses.

Chlorhexidine is a bis-biguanide formula with cationic properties. The molecule is symmetric with two 4, chlorophenyl rings and two biguanide groups linked by a hexamethylene chain. As a disinfectant called Hibitane, the drug was introduced more than 20 years ago.

Chlorhexidine has an affinity for bacteria probably due to the positively charged chlorhexidine molecule and the negatively charged groups on the bacterial cell wall. Binding increases permeability of the cell wall and permits the agent to penetrate into the cytoplasm and cause death.

Clinical studies:

Loe and Schiott (1970) originally reported that two daily rinses with 0.2% chlorhexidine almost completely inhibited plaque formation and prevented gingivitis in their model. Other studies have confirmed their findings, but these trials have been of short duration.

Flotra et al. (1972) reported substantial reduction in the amount of plaque and gingivitis in 50 soldiers who used chlorhexidine and tooth brushing for 50 months. Flora stated, however, that established periodontitis was not influenced i.e. subgingival plaque was unaffected. A number of reports have shown the affinity of chlorhexidine for proteins, bacteria and extracellular polysaccharides of bacterial origin. During a mouthrinse, chlorhexidine molecules immediately bind to acidic macromolecules on oral surfaces and are retained there. From these areas of retention, the drug is gradually released and the concentration of chlorhexidine in the mouth is kept on a level sufficient to create a bacteriostatic environment for a prolonged period of time.

Chlorhexidine rinses kill only about 80% of the bacteria in the saliva. Moreover, two daily mouth rinses are capable of keeping the relatively low level of variable microorganisms in the saliva fairly constant. When chlorhexidine usage is terminated, the number of bacteria returns to pre-experimental values within 48 hours. Chlorhexidine has been reported to be effective against Candida albicans in vitro and in vivo (denture stomatitis). There is no evidence that chlorhexidine is permanently retained in the body. The drug may penetrate the oral mucosa, but the amounts are probably very small. Some of the more serious side effects reported are: interference of taste sensation, staining, mucosal desquamations and possible interference with anti-viral mechanisms. Side effects increase with increasing use of the drug.

Disinfection, Sterilization, and Preservation

Fourth Edition

SEYMOUR S. BLOCK, Ph.D.
Professor Emeritus of Bioengineering
Department of Chemical Engineering
University of Florida
Gainesville, Florida

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CHAPTER 16

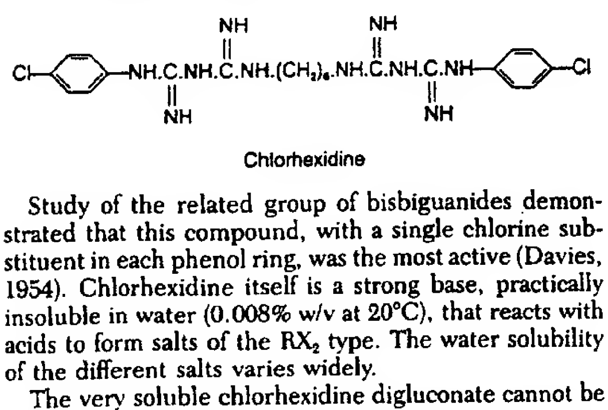
CHLORHEXIDINE

Graham W. Denton

Chlorhexidine was first synthesized in 1950 in the laboratories of ICI England during antimicrobial research into synthetic antimalarial agents of the proguanil-type. It was found to possess a high level of antibacterial activity, low mammalian toxicity, and a strong affinity for binding to skin and mucous membranes. These properties led to the development of chlorhexidine principally as a topical antiseptic for application to such areas as skin, wounds, and mucous membranes, and for dental use. In addition, chlorhexidine has been used as a pharmaceutical preservative, particularly in ophthalmic solutions and as a disinfectant for items such as inanimate surfaces and instruments.

CHEMISTRY

Chlorhexidine is 1,6-di(4-chlorophenyl-diguanido) hexane, a cationic bisbiguanide of the following formula:



Chlorhexidine

Study of the related group of bisbiguanides demonstrated that this compound, with a single chlorine substituent in each phenol ring, was the most active (Davies, 1954). Chlorhexidine itself is a strong base, practically insoluble in water (0.008% w/v at 20°C), that reacts with acids to form salts of the RX_2 type. The water solubility of the different salts varies widely.

The very soluble chlorhexidine digluconate cannot be isolated as a solid and is manufactured as a 20% w/v aqueous solution (Chlorhexidine Gluconate Solution BP), higher concentrations being too viscous for convenient use. The diacetate salt has a solubility of 1.9% w/v (20°C), whereas the dihydrochloride and other inorganic salts are relatively insoluble (Table 16–1).

The low solubility of the inorganic salts may cause problems of precipitation if a water-soluble salt such as digluconate is formulated with, or diluted in, a solution containing inorganic anions such as sulphate or carbonate.

Generally, the solubility of chlorhexidine salts in alcohol is higher than that in water; however, chlorhexidine gluconate solution should not be added directly to neat alcohol, because precipitation may occur.

Solutions and powders of chlorhexidine are colorless or almost colorless and usually odorless, although formulations prepared from the diacetate salt occasionally have an odor of acetic acid. Solutions prepared from all salts have an extremely bitter taste that must be masked in formulations intended for oral use.

Chlorhexidine is moderately surface-active and forms micelles in solution; the critical micellar concentration of the acetate is 0.01% w/v at 25°C (Heard and Ashworth, 1968). Aqueous solutions of chlorhexidine are most stable within the pH range 5 to 8. Above pH 8.0 chlorhexidine base is precipitated, and in more acid conditions there is gradual deterioration of activity because the compound is less stable. Hydrolysis yields p-chloroaniline; the amount is insignificant at room temperature, but is increased by heating above 100°C, especially at alkaline pH (Goodall, 1968).

Chemical analysis of chlorhexidine preparations may be performed using a variety of different methods. Samples containing 1 g or more of chlorhexidine may be assayed by the method described in the British Pharmacopoeia, i.e., by dissolving the evaporated residue in glacial acetic acid (neutralized to crystal violet) and titrating against perchloric acid potentiometrically, using a glass electrode. For lower concentrations, a colorimetric method may be used, involving a reaction with alkaline sodium hypobromide, which produces a reddish-brown color (Holbrook, 1958). Chlorhexidine can also be analyzed by gas liquid chromatography (Siefert, 1975) and high-performance liquid chromatography (Huston, 1982; Richard, 1984).